Ubiquitin-Positive Pneumocytes and Inclusion Bodies are Present in Secondary Organizing Pneumonia

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Abstract

Objective  We previously reported that various types of interstitial pneumonia (IP) patterns contain intracytoplasmic eosinophilic inclusions or Mallory bodies (inclusions) that are ubiquitin positive (Ub+). In the present study, we examined tissues with the organizing pneumonia pattern (OP) to determine if they contain inclusions and Ub+ pneumocytes using lobectomized specimens.

Methods  Tissues from 34 patients with secondary OP, which appeared in 33 carcinomas and 1 pulmonary abscess, were histologically evaluated for the type of intraluminal granulation tissue and the presence of erosions and inclusions. Granulation tissues were classified into polypoid, mural and occluded subtypes according to Basset’s criteria and scored.

Results  Inclusions were noted in 5.9% of the secondary OP cases with light microscope. Ub+ pneumocytes were detected after immunostaining and all inclusions were Ub+. Ub+ pneumocytes (inclusions) were noted in 14.7% of the secondary OP cases. OP contained pneumocyte erosions and inflammatory cell infiltration without a significant difference in the Ub+ and Ub- subgroups. Although there was no significant difference in the polypoid type of granulation tissue between the Ub+ and Ub- negative (Ub-) subgroups, the Ub+ subgroup had significant increases (p<0.05) in the mural-occluded type of granulation tissue (Ub+: 1.76 ± 0.64, n=5 vs. Ub-: 0.72 ± 0.87, n=29) as compared to the Ub- subgroup.

Conclusion  Some patients with secondary OP had Ub+ inclusions as pneumocyte injury.

Key words: secondary organizing pneumonia, ubiquitin, inclusion body, pneumocyte injury


Introduction

Ubiquitin (Ub) plays a key role in proteolysis through the ubiquination of target proteins (1, 2) and the Ub gene is expressed in all eukaryotic cells (3, 4), but, Ub positive (Ub+) cells have been found in only a few visceral organs and in some tumors (5-7). We have identified Ub+ pneumocytes in cases of lung disease (8). Inclusion bodies (inclusions) have been found as hyaline globules or spherical intracytoplasmic eosinophilic droplets in patients with a diffuse alveolar damage pattern (DAD) or asbestosis (9-11). The inclusions were found to be Ub+ by immunohistochemistry (12). We reported the relationship between the accumulation of Ub in pneumocytes from individuals with DAD and the degree of hyaline membrane formation. We previously studied the usual interstitial pneumonia pattern (UIP), which had a poor prognosis among interstitial pneumonia (IP) and the nonspecific interstitial pneumonia pattern (NSIP) to examine the relationship between Ub+ pneumocytes and pneumocyte injury. In the present report, utilizing lobectomized specimens we examined the organizing pneumonia pattern (OP), which has a better prognosis, to know whether Ub+ pneumocytes and inclusion are associated with pneumocyte injury.

Methods

We studied 34 patients with secondary OP that was found...
at the periphery of lobectomized lung cancer (30 men and 4 women; mean age, 66.4 years; range, 49–80 years). Cryptogenic OP (COP) was excluded in this study. All procedures were performed at the Saitama Cardiovascular and Respiratory Center (Kumagaya City, Japan). Lung pathologists histologically diagnosed OP based on the criteria of the American Thoracic Society/European Respiratory Society consensus classification system (ATS/ERS) (13). There was no history of irradiation and no patients were administered anti-carcinoma agents before lobectomy. Nine of the patients were diagnosed as adenocarcinoma; 21 squamous cell carcinomas; 2 neuroendocrine carcinomas; 1 metastatic urothelial carcinoma; and 1 non-specific pulmonary abscess.

Light microscopy:

Secondary OP was prepared for histological analyses from lobular or confluent consolidated areas apart from and along the periphery of lung tumor or abscess in the study. Samples for lung histology from lobectomy were embedded in paraffin after 20% formalin fixation under the proper conditions. Then, 4-μm sections from all samples were prepared. The sections were stained with Hematoxylin-Eosin (HE) and evaluated for inclusions, inflammatory cells, pneumocyte injury, and fibrosis. The mean observed area of the lung specimens was 4.0 cm² per section. Only inclusions with a distinct border in the pneumocyte cytoplasm were counted, and indistinct type with HE staining was excluded.

Immunohistochemistry:

Formalin-fixed lung tissue sections were stained with polyclonal antibodies to Ub (diluted 1:50, Sigma, Lot No U-5379), monoclonal antibodies to cytokeratin KL-1 (diluted 1:50, Abcam), and cytokeratin AE1/AE3 (diluted 1:50, IMGENEX) for inclusions. Epithelial membrane antigen (EMA, diluted 1:200, Dako) was immunostained to confirm erosion. The sections were immunohistochemically stained according to the manufacturer’s protocol and the previous report (8). When inclusions were found by light microscopy, the adjacent sections were immunohistochemically stained for Ub to compare the HE-stained inclusions with the Ub staining. To precisely compare the HE and Ub staining characteristics of the inclusions, we initially photographed HE-stained inclusions. We then removed the cover glass slides from the HE-stained sections, immunostained them for Ub after decolorization of the HE staining, and photographed the Ub-stained sections. We compared the photographs of the HE and Ub staining.

Semiquantitative morphometric analyses:

We evaluated the number of inclusions and the number of Ub-positive pneumocytes. We scored and averaged the degree of inflammatory cell infiltration in each section (grade 0: no inflammatory cells; grade 1: mild inflammatory cell infiltration; grade 2: moderate; and grade 3: severe), and compared scores between Ub+ and Ub-negative (Ub−) groups. Pneumocyte injury or its related histological feature was indicated by the presence of erosions, which appeared as a complete defect or disappearance of pneumocytes next to alveolar interstitium. The erosions were counted in the lower power fields, and the mean number per case was determined as a grade for cellular injury. The granulation tissues due to pneumocyte injury were histologically classified according to the methods of Basset, et al (Fig. 1) (14) into 3 subtypes based on histological characteristics: intraluminal bud (or polypoid type), mural incorporation (mural type), or obliterator changes (occluded type). Each granulation tissue subtype was scored as grade 0 to 4 (grade 0: no granulation or each subtype, grade 1: less than 24%; grade 2: 25%-49%, grade 3: 50%-74%, grade 4: over 75%) due to their numbers and expansion with low-power field in each lung section, respectively. We analyzed the granulation tissue subtypes in this study, divided into 2 groups; polypoid type and mural-occluded type according to mild or severe pneumocyte injury. Total scores of the polypoid type and mural-occluded type were calculated and then averaged.

Data are expressed as mean ± SD. Differences between the groups were evaluated by using Student’s t test, p-values of less than 0.05 were considered statistically significant. The study was approved by a local hospital review board for human studies and patients gave informed consent to participate in the study prior to the surgery, in accordance with the Declaration of Helsinki.

Results

Light microscopy:

The histopathological findings of the OP group are summarized in Table 1 and shown in Fig. 2. The OP contained inclusions and the shape of inclusions varied from eosinophilic filamentous to globular, with a distinct border in the pneumocyte cytoplasm (Fig. 3, left). Among the 2 patients (5.9%) with inclusions, the mean number of inclusions was 1.5 per section (Table 1). The inclusions were noted in a patient with squamous cell carcinoma or neuroendocrine carcinoma.

Immunohistochemistry:

Ub+ pneumocytes were detected (Fig. 3, right) in 14.7% among the OP. Serial sections showed that the inclusions were Ub+ in the same pneumocytes. Among the 5 (14.7%) patients with Ub+ pneumocytes, the mean number of Ub+ pneumocytes was 4.6 per section (Table 1). The number of Ub+ pneumocytes detected immunohistochemically was greater than the number of inclusions. Furthermore, the identical inclusions (Fig. 4, left) were Ub+ using the same specimen after decolorization of the HE-stained section (Fig. 4, right). All inclusions found with the HE-stained sections were Ub+ in the studied cases. Pneumocytes and inclusions were positive for KL-1, and the inclusions were infrequently positive for AE1/AE3. The Ub+ pneumocytes were located adjacent to the granulation tissue.
Figure 1. Granulation tissue subtypes and erosion: Photographs show representative histological characteristics of intraluminal granulation tissue subtypes with the same magnification; polypoid type in upper left panel (arrow, Hematoxylin and Eosin staining), mural type in the upper right panel (arrows, Hematoxylin and Eosin staining), and occluded type in the lower left panel (arrow, Weigert’s van Gieson staining). The lower right panel (Hematoxylin and Eosin staining) shows erosion in the alveolar wall with a complete defect of pneumocytes. Bar, 100 μm.

Table 1. Summary for Inclusions in Organizing Pneumonia

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>34 cases</th>
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<tbody>
<tr>
<td>male (cases)</td>
<td>30 cases</td>
<td></td>
</tr>
<tr>
<td>female (cases)</td>
<td>4 cases</td>
<td></td>
</tr>
<tr>
<td>mean age at surgery or biopsy</td>
<td>66.4 year-old</td>
<td></td>
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<table>
<thead>
<tr>
<th>Light microscope</th>
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<tbody>
<tr>
<td>inclusions+</td>
<td>2 cases</td>
</tr>
<tr>
<td>rate for inclusion+ case</td>
<td>5.9 %</td>
</tr>
<tr>
<td>mean number of inclusions</td>
<td>1.5</td>
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</table>

<table>
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<th>Immunostaining</th>
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<tr>
<td>Ub+ inclusion cases</td>
<td>5 cases</td>
</tr>
<tr>
<td>rate for Ub+ case</td>
<td>14.7 %</td>
</tr>
<tr>
<td>mean number of Ub+ cells</td>
<td>4.6</td>
</tr>
</tbody>
</table>

Cells that contained inclusions were identified by light microscopy and counted. Ub+ cells were identified by immunohistochemistry and counted. Ub: ubiquitin.

The patients with Ub+ subgroup contained all types of carcinoma included 2 cases of adenocarcinomas, 1 squamous cell carcinoma and 2 neuroendocrine carcinomas in this study. No correlation was found between the Ub+ pneumocyte and histological type of carcinoma in the cases with secondary OP.

Semiquantitative analyses:

Histological characteristics of the erosion and granulation tissue subtypes are shown in Fig. 1. Erosions were found in 35.3% (12 cases) of the OP; 8.8 % in the Ub+ subgroup and 26.5 % in the Ub-negative (Ub-) subgroup. There was
Patients with OP had a preferable prognosis, while, some patients with the secondary OP had inclusions in pneumocytes, these inclusions were proved to be Ub+. The Ub+ pneumocytes were confirmed to be the same as the inclusions by immunostaining of decolorized HE sections. The identical inclusions were Ub+ using the same sections. Ub+ inclusions have been considered to be a histological marker for pneumocyte injury in the previous studies (8, 15), and the secondary OP group had the lowest rate of Ub+ cases among the IPs reported (rate for inclusion: 40.7% of DAD cases, 74.3% of UIP cases, 21.4% of NSIP cases; rate for Ub+: 48.1% of DAD, 80.0% of UIP, 39.3% of NSIP) (15, 16). The UIP and DAD showed high positive rates and increased numbers of inclusions and Ub+ pneumocytes, proving the pneumocytes were most severely injured. NSIP showed intermediate data and the secondary OP showed mild pneumocyte injury among the IPs. However, the secondary OP with increased mural and occluded types of granulation tissue showed a relatively higher positive ratio, suggesting that the different prognosis and therapeutic response of IPs might be affected by different degrees of pneumocyte injury. Rates for the inclusion and Ub+ in the patients with OP were small, but the Ub+ subgroup had a significantly higher score in mural-occluded type granulation tissue than the Ub- subgroup, which was similar to IPs studied previously. Ub+ had a relation to intraluminal organization due to pneumocyte injury.

Pneumocyte injury and its related lesions (exudation, erosions, inclusions, and Ub+ pneumocytes) were evaluated by light microscopy, with analysis of OP which appeared in the lobectomized specimen. We did not analyze the cause of pneumocyte injury, but the cases were considered to be probable bacterial infection. Thus bacterial pneumonia and its sequence were suggested to induce pneumocyte injury and erosion in the OP. Pneumocyte injury induced an accumulation of Ub-proteins in pneumocyte cytoplasm, because proteasome-proteolysis was not in progress in damaged pneumocytes. The Ub-proteasome system (UPS) has a greater role in disorders of many organs (17, 18). Increased accumulations of Ub-proteins have been detected as inclusions with a light microscope. Ub immunostaining had a high sensibility for detection of inclusions as compared to light microscopic detection (Table 1), and Ub enhanced detection of inclusions especially in the questionable cases. Ub+ pneumocytes included the inclusion and the indistinct type of inclusion which was excluded with HE staining. Ub-proteins were accumulated through inhibition of UPS (19) and appeared as inclusions. Ub+ was found in inclusions (20-22), and cellular injury was also reported to inhibit UPS (23).

Ubiquitination of protein is reversible and deubiquitination is catalyzed by deubiquinating enzymes (24, 25). The inclusions have been reversible histological features, while,
severe pneumocyte injury induced erosion of pneumocyte after necrosis or irreversible damage. Although there was no significant increase of erosion among the patients with secondary OP in this study, erosions induced intraluminal organization or granulation tissue subtypes according to the severity of pneumocyte injury (14). In fact, the mural and occluded types of granulation tissues due to severe pneumocyte injury were more increased in the Ub+ subgroup than in the Ub- subgroup (Fig. 5). These findings suggested the presence of pneumocyte injury even in some patients with the secondary OP. The Ub+ subgroup showed a trend in that the OP lesion had a circumferential extent around lung carcinoma, compared to the Ub- subgroup, without a significant difference in the lesion extent as background. There was also no difference in the pattern of consolidation (lobular or confluent) between the subgroups. Inclusion and Ub+ pneumocyte did not correlate with the subtype of carcinoma as the patients with Ub+ subgroup had all types of carcinoma with the secondary OP. Ub+ pneumocyte was not induced by anti-carcinoma agent in these cases, because there was no administrative history of anti-carcinoma agent for all of the cases. After intraluminal granulation tissue subtypes were formed due to mild or severe pneumocyte injury, each granulation tissue subtype even in the polypoid type was suggested to have repeated pneumocyte injury, or severe pneumocyte injury at a time in the OP development. Subsequently, some pneumocytes came to have Ub+ inclusion in the cytoplasm.

UIP and NSIP frequently had the mural type and occluded type of granulation tissue, and higher rates for inclusions and Ub+ (15, 16). OP with intraluminal organization such as mural and occluded type of granulation tissues had increased rates for inclusions and Ub+ pneumocytes, similar to UIP and NSIP, but, the studied cases remained in a category of OP. While, the polypoid type due to mild injury had no relation to the inclusion and Ub+ pneumocytes. A different prognosis was suggested between OP patients with the mural-occluded type and the polypoid type. The studied OP cases were obtained from surgical specimens diagnosed as lung carcinoma, therefore, an evaluation for prognosis or therapeutic responses among OP was not enough in this study. Ub+ pneumocyte was uncertain as a prognostic factor.

Pneumocyte injury induces necrosis or the erosion of pneumocytes, each subtype of granulation tissue, and the inclusions that are features of the accumulation of Ub-proteins. DAD, UIP, and NSIP have the inclusions and Ub+ pneumocytes, and the Ub+ is not a specific feature among the IPs. Our data suggest that the Ub+ cells can be a marker of pneumocyte injury in the lung. Additionally, there was pneumocyte injury adjacent to the granulation tissues during the OP development. Finally, the Ub+ pneumocytes are present even in some patients with OP, due to pneumocyte in-
Scores for inflammatory cell infiltration and granulation tissue subtype in organizing pneumonia

![Scores for inflammatory cell infiltration and granulation tissue subtype in organizing pneumonia](image)

**Figure 5.** Scores for inflammatory cell infiltration and granulation tissue subtype in organizing pneumonia: There is a significant increase in the mural-occluded type of granulation tissues in the Ub+ subgroup, without a significant increase in the polypoid type granulation tissue, as compared to the Ub- OP subgroup. A higher tendency of inflammatory cell infiltration score is noted in the Ub+ subgroup of the patients with secondary OP compared to the Ub- subgroup, without a significant difference.

The authors state that they have no Conflict of Interest (COI).

### References


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